Significance of Digging Behavior to Mortality of Red Imported Fire Ant Workers, *Solenopsis invicta*, in Fipronil-Treated Sand

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ABSTRACT The effect of fipronil-treated sand on digging behavior and mortality of red imported fire ant, *Solenopsis invicta* Buren, workers was examined in the laboratory. No-choice digging bioassays where fipronil-treated sand was the only available digging substrate were conducted on two colonies at fipronil concentrations of 0.00, 0.05, 0.10, 0.50, 1.00, 1.50, and 2.00 ppm. Workers dug into the fipronil-treated sand in all cases, even at 2.0 ppm level, which caused 100% mortality in acute toxicity tests for both colonies. At 1.5 and 2.0 ppm, workers from the less sensitive colony had significantly higher mortality than those from the more sensitive colony, which might be explained by the significantly higher digging activity of the less sensitive colony. In two-choice digging bioassays where untreated sand was also available, workers dug into the fipronil-treated sand in 29 of 30 cases, even at 10.0 ppm level. At 1.0 and 10.0 ppm, mortality was positively correlated to digging effort in treated sand; however, such correlation was significant only at 1.0 ppm level. This indicates that digging did affect mortality; however, such effect is concentration dependent.

KEY WORDS digging behavior, mortality, fipronil, fire ant

The social structure of ants dictates that most species construct nests. The majority of ant species build their nests in soil. Digging behavior is critical to nest construction and establishment of red imported fire ants, *Solenopsis invicta* Buren, hereafter referred to as fire ants. A monogyne fire ant colony begins with a dealated queen that digs her nuptial chamber after a successful mating flight. Budding, one reproductive strategy of polygyne fire ants (Vargo and Porter 1989), also requires digging, a process of building new nests in soil. In the laboratory, workers of both monogyne and polygyne social forms, always show digging behavior whenever adequate substrate is available. This behavior is essential not only in building, enlarging, and repairing nests but also in constructing foraging tunnels (Markin et al. 1975). Digging behavior has long been a subject of ant research (Wilson 1958; Sudd 1969, 1971, 1972, 1975; Deneubourg and Franks 1995; Mikheyev and Tschinkel 2004). Energy and time that ants spend in digging can be substantial. In a laboratory study, Sudd (1969) studied conditions in which a single ant from 16 different species would dig and the specific movements used. He estimated that a worker might expend 10% of its daily energy intake on nest excavation. Mikheyev and Tschinkel (2004) studied the architecture of underground nests of *Formica pallidefulva* Latreille and the costs and rules of nest building. They found that if a colony moved twice a year, it would expend 21% of its energy intake and 6% its worker time on nest excavation.

Many biological and environmental factors influence ant digging behavior. Soil moisture is important for mound-building activity of *S. invicta* (Rhoades and Davis 1967). They observed that mound building of fire ants increased after a rain. Carbon dioxide is a releaser of digging behavior of *Solenopsis geminata* F. (Hangartner 1969). Hubbard (1974) found when given a choice, *S. invicta* workers dug preferentially in their own nest material. Rasse and Deneubourg (2001) noted a positive correlation between the number of *Lasius niger* F., and digging rate as well as final volume of the nest.

Although digging is almost certainly an innate behavior, it has never been investigated for the manipulation of this behavior in ant management. One potential scenario, in which digging behavior would have positive impact on fire ant management, is the use of contact insecticides, such as individual mound treatment. If the insecticide was nonrepellant and slow acting, workers would dig into treated mound soil, attempting to repair the collapsed mound. Such behavior would increase the chance for ants to contact insecticide and improve insecticide efficacy. Individual mound treatment and broadcast application are common practices in fire ant management (Morrill 1977; Francke 1983; Williams and Lofgren 1983); how-

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ever, no information is available on the relationship between digging behavior and efficacy.

The objective of this study was to investigate the relationship between fire ant digging behavior and mortality in fipronil-treated sand. Fipronil has been used as both contact insecticide and bait toxicant in control of fire ant (Collins and Callcott 1998, Greenberg et al. 2003, Barr and Best 2004) and Argentine ant, *Linepithema humile* (Mayr) (Costa and Rust 1999, Hooper-Bui and Rust 2000). It has been tested as bait toxicant against *Lasius neoniger* Emery (Lopez et al. 2000); *Tapinoma melanocephalum* (F.) (Ulloa-Chacón and Jaramillo 2003); and the Texas leafcutting ant, *Atta texana* (Buckley) (Grosman et al. 2002). Successful application of fipronil as a bait toxicant indicates that fipronil is not repellant to fire ants, at least at the concentration levels used.

The following approaches were used to test the hypothesis that the innate behavior of digging will enhance fipronil efficacy: 1) determining sensitivities of worker ants from two different colonies to fipronil in sand by estimating their LC_{50} values, 2) comparing the digging behavior and mortalities of these two colonies in no-choice bioassays where the fipronil-treated sand was the only available digging substrate, and 3) establishing correlations between digging behavior and mortalities within a colony in two-choice bioassays where untreated sand was available.

Materials and Methods

Insects. Four red imported fire ant colonies were collected from Sharkey County, Mississippi: two on 15 November 2004 and the other two on 23 February 2005. Colonies were separated from soil by using a method of Banks et al. (1981), except a higher water dropping rate, 40 drops per min, was used instead of 20 drops per min. Alates were found in all colonies, indicating they were mature colonies. Ants were reared in a plastic tray (44.5 by 60.0 by 13.0 cm) in which the inside wall was coated with Fluon (Ag Fluoropolymers, Chadds Ford, PA) to prevent ants from escaping. Distilled water was supplied in a 500-ml polypropylene square bottle (VWR International, West Chester, PA) that was laid down in the tray and had two holes (2.0 cm in diameter) on each of its two lateral sides. The holes were blocked using cotton balls that gave ants free access to the water. A 14- by 2.0-cm petri dish with 10-cm-thick bottom layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA) was used as an artificial nest in the tray. At the center of the petri dish was a 5.0-cm-diameter brood chamber. Access holes (8 mm in diameter) were located on the wall of the petri dish above the dental plaster. Cotton bollworm, *Heliocoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), pupae were used as food sources and provided ad libitum in a petri dish. Colonies were maintained in a rearing room at 25°C, 58% RH, and a photoperiod of 12:12 (L:D) h.

All four colonies were monogyne, which was determined with polymerase chain reaction (PCR). Primers described in Valles and Porter (2003) were used to amplify *Gp*-9 alleles, indicating monogyne or polygyne colony status. Specimens of worker ants were collected in 70% isopropanol. Twenty or fewer ants were removed from isopropanol and air-dried and then used as a single sample for genomic DNA extraction. Genomic DNA was extracted using the Promega Wizard SV genomic DNA purification system according to manufacturer's instructions for preparation of mouse tail and tissue lysates. Samples were crushed in digestion solution containing proteinase K, incubated overnight, and then centrifuged and separated from undigested materials before column application. Nuclease-free water was heated to 65°C before elution for improved yield. Samples were measured spectrophotometrically and if determined to be low yield or contaminated with protein (260/230 reading <1.0), samples were cleaned and concentrated using Zymo DNA Clean & Concentrator-5 columns. Primers were used in pairs rather than as multiplexed PCR to improve resolution. PR was conducted in a PTC 220 (MJ Research, Waltham, MA) using the following cycling parameters, after a hot start: one cycle at 95°C for 3 min and then 35 cycles at 94°C for 15 s, 55°C for 15 s, and 68°C for 30 s, followed by a final elongation step for 5 min at 68°C. Amplified DNA was visualized on a 1% agarose gel containing ethidium bromide after electrophoresis for 40 min at 90 V. Samples that produced amplicons from only one set of primers were scored as monogyne (BB homozygous) and those producing amplicons from both sets of primers (Bb heterozygous) were scored as polygyne. Both positive (polygyne) and negative (no DNA) controls were processed along with samples.

Worker LC_{50} to Fipronil in Sand. The LC_{50} values were determined by using 24-h mortality of 50 workers in a petri dish (9 cm in diameter, 2 cm in height) with 40 g of fipronil-treated sand (Play sand, Sims Bark Co., Inc., Tuscumdia, AL) with 8.0% moisture content. The moisture content was adjusted by adding 17.39 ml of water into 200 g of sand in the beaker and stirring the sand with a glass rod for 2 min to ensure mixing of water with sand. The inside wall of petri dish was coated with Fluon to ensure that all ants contacted sand when they were placed in the petri dish. Tested concentrations of fipronil were 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0 ppm. A fipronil (analytical standard, purity 97.5%, Sigma, St. Louis, MO) solution was prepared using acetone as solvent. Treated sand was prepared by pouring a 10-ml fipronil solution into 200 g of sand in a 1000-ml beaker and then shaking the beaker to mix. Acetone was evaporated under a fume hood for 40 min. A control was set up by using sand treated with 10 ml of acetone. Sand moisture content was adjusted to 8% as noted above. Two different colonies (A and B) were tested. Worker sizes of colony A and B, based on the measurement of 40 randomly sampled workers of each colony, were 1.28 ± 0.93 mg (mean $\pm$ SD; minimum size, 0.6 mg; maximum size, 4.6 mg) and 1.43 ± 1.14 mg (minimum size, 0.5 mg; maximum size, 5.0 mg), respectively. Workers were randomly sampled, which was achieved by using the following pro-

cedure: one end of a glass rod was placed inside an opened artificial nest for ≈5 s, workers climbing on the rod were transferred into a plastic cup coated with Fluon, and ants were counted. The procedure was repeated until 50 workers were sampled. Workers were then placed in each petri dish. There were five replicates on each concentration for each colony. The means of percentage of mortality were subjected to probit analysis to estimate LC_{50} values (SAS Institute 1999). Ant mortality among treatments was compared using GLM analysis of variance (ANOVA) followed by a least significant difference (LSD) test (PROC GLM, SAS Institute 1999). Significance was accepted at $P < 0.05$.

No-Choice Digging Bioassays. The bioassay apparatus consisted of one petri dish (9 by 2 cm) and a capped Wheaton liquid scintillation vial (2.8 by 6.1 cm) centered underneath it. A 3-mm access hole was drilled through the center of a petri dish, and the vial cap underneath and the inside wall of the petri dish was coated with Fluon. Sand preparation method for treatment was the same as described above. Sand in the control vial was treated only with acetone. Seven concentrations of fipronil were tested: 0.00, 0.05, 0.10, 1.00, 1.50, and 2.00 ppm. A mean ± SD of 36.80 ± 0.8 g of sand was added into each vial. Workers were sampled using method described as above. One hundred fire ant workers were introduced into each petri dish. After 24 h, sand in each vial was collected, dried at 250°C for 12 h, and weighed. Colonies A and B were used for this experiment. For each colony, there were 10 replicates for each concentration. A t-test (critical P value = 0.05) was used to compare mean amount of sand removed and ant mortality for each concentration between two colonies. A completely randomized design was used and a GLM ANOVA followed by an LSD test (PROC GLM, SAS Institute 1999) was used to compare the amounts of removed sand and ant mortality among and between treatments. Significance was accepted at $P < 0.05$.

Two-Choice Digging Bioassays. The bioassay apparatus consisted of three petri dishes (9 by 2 cm), which were connected using glue (Arrow Fastener Co., Inc., Saddle Brook, NJ). Each dish was glued to a capped Wheaton liquid scintillation vial (2.8 by 6.1 cm), which was right under the petri dish. They were referred to as home dish, treatment dish, and control dish, respectively. In the treatment and control dishes, a 3-mm access hole was drilled through the center of each dish and the vial cap underneath it, whereas the home dish had no access hole. There were holes on the connection points between the home dish and the other two dishes. The inside wall of each petri dish was coated with Fluon. Sand preparation and worker sampling methods were the same as those described above. Workers were sampled using the method described above. One hundred fire ant workers were introduced into each home dish. Three concentrations of fipronil, 0.1, 1.0, and 10.0 ppm, were tested, and the experiment was conducted at 22 ± 0.45°C. After 24 h, sand in each dish was collected, dried at 250°C for 12 h, and weighed. Sand was collected from each vial if sand was found in the home dish, and the amount of sand removed was determined from the weight difference before and after. Ant mortality was recorded for each apparatus. Two colonies (C and D) were used for this experiment. Worker sizes of colony C and D, based on the measurement of 40 randomly sampled workers of each colony, were 1.20 ± 0.67 mg (mean ± SD; minimum size, 0.7 mg; maximum size, 4.0 mg) and 1.28 ± 0.87 mg (minimum size, 0.5 mg; maximum size, 4.0 mg), respectively. For each colony, there were 10 replicates for each concentration. A paired t-test (critical P value = 0.05) was used to compare the mean amount of sand removed from a treated vial with that from a control vial for each concentration. SAS (PROC CORR, SAS Institute 1999) was used to calculate the Pearson correlation coefficient, which measures the correlation between amounts of sand removed from treated vial and ant mortality.

Results

Worker LC_{50} to Fipronil in Sand. The LC_{50} for colony A was 0.27 ppm (95% fiducial limits [FL], 0.24–0.31 ppm) and 0.51 ppm for colony B (95% FL, 0.48–0.54 ppm) (Fig. 1). Workers from colony B are less sensitive to fipronil than those from colony A. At 2.0 ppm, 100% worker mortality was achieved for both colonies. Although fipronil had a significant effect on

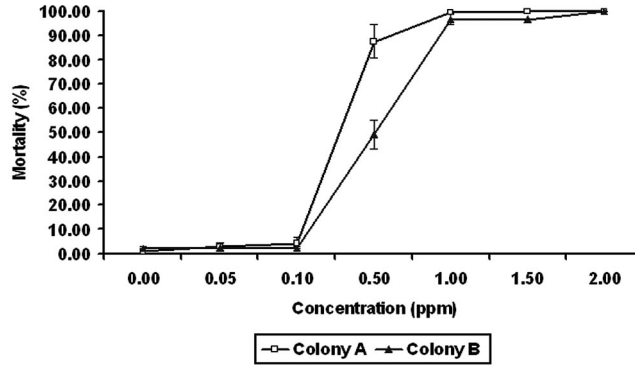

Fig. 1. Mean percentage of mortality (±SE) ($n = 5$) of *S. invicta* workers from two colonies in fipronil-treated sand.

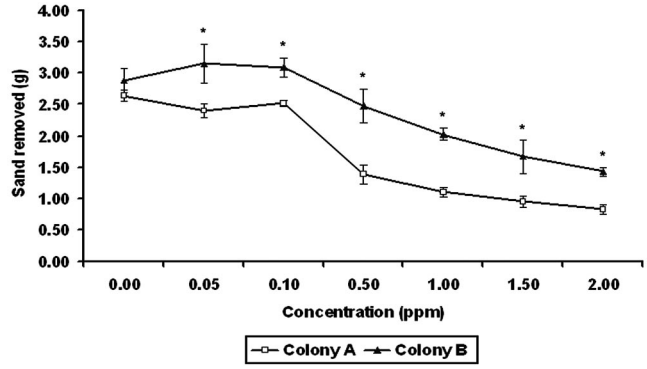

Fig. 2. Mean amount of sand removed (±SE) ($n = 5$) in no-choice bioassays for colony A and B. *, significantly different between two colonies ($P < 0.05$).

ant worker mortality for both colony A ($F = 322.6$, df = 6, $P < 0.0001$) and colony B ($F = 395.9$, df = 6, $P < 0.0001$), mortality at concentrations of 0.05 and 0.10 ppm was not statistically different from that of the control.

No-Choice Digging Bioassays. Amounts of removed sand and ant mortality are summarized in Figs. 2 and 3 for colony A and B. Workers dug into all sand in treatments. In controls, the amounts of removed sand were not significantly different between colony A and B; however, for each concentration level, colony B removed more sand than colony A. There was a significant effect of fipronil on the amount of sand removed from vials for both colony A ($F = 72.29$, df = 6, $P < 0.0001$) and colony B ($F = 10.67$, df = 6, $P < 0.0001$). The effect of 0.05 and 0.10 ppm on sand removal was not statistically different from control for both colony A and B. For colony B, the difference between 0.5 ppm and control was also not statistically significant. Worker mortality of colony B was significantly higher than that of colony A at 1.5 ppm ($t = -4.76$, df = 8, $P = 0.0014$) and 2.0 ppm ($t = -3.17$, df = 8, $P = 0.013$).

Two-Choice Digging Bioassays. Amounts of removed sand and ant mortality are summarized in Fig. 4. Workers dug into all fipronil-treated sand with only one exception. For colony C, at 0.1 ppm, there was no significant difference on amount of sand removed from control and treatment vial ($t = -0.92$, df = 9, $P = 0.38$); however, significantly less sand was removed from fipronil-treated vials than from control vials at 1.0 ppm ($t = 3.03$, df = 9, $P = 0.014$) and 10.0 ppm ($t = 3.71$, df = 9, $P = 0.049$). For colony D, significantly less sand was removed from fipronil-treated vials than from control vials, at 0.1 ppm ($t = 4.58$, df = 9, $P = 0.0013$) and 1.0 ppm ($t = 5.59$, df = 9, $P = 0.0003$); however, at 10.0 ppm level, such a difference was not statistically significant ($t = 1.10$, df = 9, $P = 0.3$). Worker mortality never reached 100%, even at 10.0 ppm level. At 1.0 ppm, amounts of sand removed from treated vials were positively correlated to worker mortality for both colony C ($r = 0.85$, $P < 0.002$; Fig. 5) and colony D ($r = 0.95$, $P = 0.002$; Fig. 5). At 10.0 ppm, such correlations were also positive for both colonies (colony C: $r = 0.55$, $P = 0.10$; colony D: $r = 0.25$, $P = 0.48$); however, they were not statistically significant for both colonies.

Discussion

In no choice bioassays, worker ants dug into fipronil-treated sand in 100% of the cases, and in two-

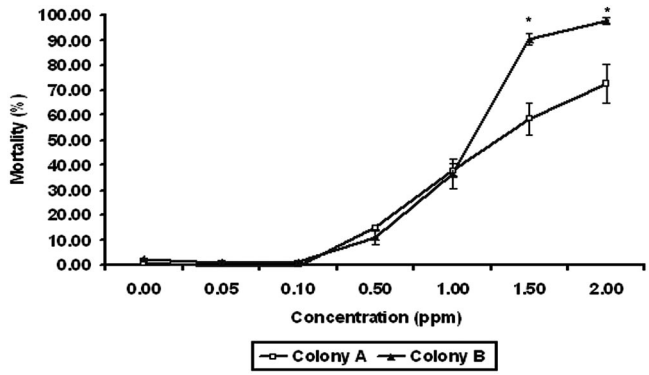

Fig. 3. Mean worker mortality (±SE) ($n = 5$) in no-choice bioassays for colony A and B. *, significantly different between two colonies ($P < 0.05$).

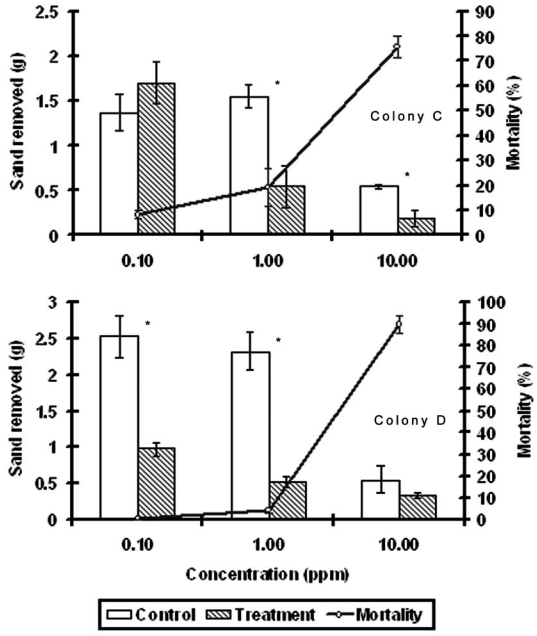

Fig. 4. Mean amount of sand removed (±SE) and worker mortalities (±SE) ($n = 5$) in two-choice bioassays for colony C and D. *, significantly different between control and treatment ($P < 0.05$).

choice bioassays where untreated sand was also available, workers excavated fipronil-treated sand in 98.33% of the cases. This indicates that fipronil was not repellant to red imported fire ant workers at the concentrations tested. Without digging behavior, fire ant workers would have minimal exposure to fipronil, because the diameter of the access holes in both no-choice and two-choice bioassays was only 3.0 mm. Digging behavior clearly gives workers a greater opportunity to contact fipronil.

Workers from colony B had a greater LC_{50} value, which indicated that they were less sensitive to fipronil than those from colony A; however, in no choice tests, at 1.5 and 2.0 ppm, workers from colony B had significantly higher mortality than those from colony A. It could only be explained by the significantly higher digging activity of colony B. Such effect did not show at concentrations of 0.1, 0.5, and 1.0 ppm, which indicated that effect of digging behavior on fipronil efficacy might be concentration dependent. Such dependence was further demonstrated in two-choice bioassays. At 1.0 and 10.0 ppm, worker mortality was positively correlated to digging effort for both tested colonies; however, such correlation was significant only at 1.0 ppm. The concentration dependence can be readily explained if fipronil concentration was conceptually extended to the extremes at both ends: 1) if fipronil concentration is low enough, such as at nonlethal level, more digging would not cause any more mortality; or 2) if concentration is too high, workers would die after the initial digging, so the effect of digging on fipronil efficacy would not be able to exhibit or not exhibit enough to statistically separate the differences among treatments.

Contact insecticides have long been used in fire ant control (Hays 1988). They have been formulated as mound drenches, granular for mound treatment and

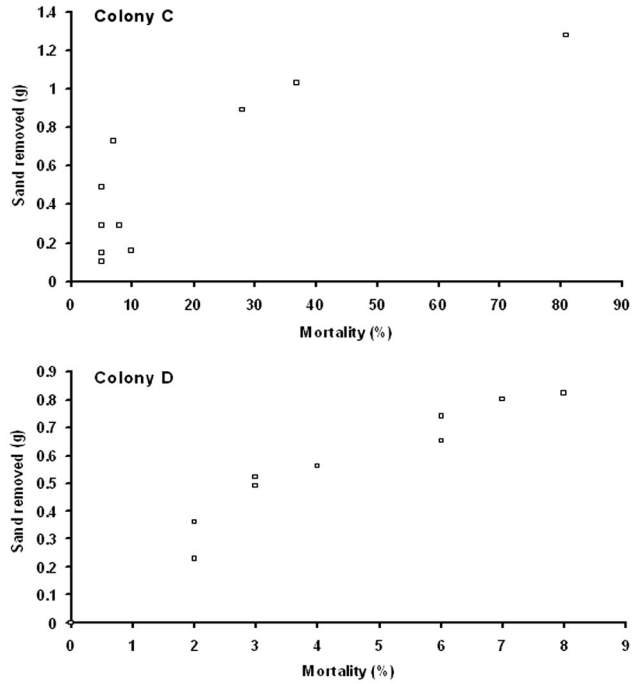

Fig. 5. Correlations between amount of sand removed from vials with 1.0 ppm fipronil-treated sand by workers and their mortalities in two-choice bioassays. r is 0.85 ($P = 0.002$) for colony C and 0.95 ($P < 0.0001$) for colony D.

broadcast, and aerosol injections. Contact between ants and contact insecticide is always a prerequisite for such treatments to be effective and measures have been developed to maximize such contact, such as using high volume of insecticide liquid to facilitate the penetration of insecticide in the mound and applying insecticides when most ants and the brood are high in the mound (Hays 1988). The results of this study showed that such contact may be enhanced by taking advantage of ant's intrinsic digging behavior without increasing the dose of insecticide.

Fipronil granular products for fire ant control are currently available on the market, such as Over'n Out (0.0103% fipronil, TechPac, LLC, Lexington, KY) and TopChoice (0.0143% fipronil, Bayer Environmental Science, Montvale, NJ). The recommended application rate for both products is 2 lb per 1,000 square feet, equivalent to 0.1006 and 0.1396 $\mu g/cm^2$ fipronil for Over'n Out and TopChoice, respectively. After being broadcast over a target area, fipronil in these products is released into the soil via rainfall or irrigation. Fire ants come in contact with fipronil when they walk on treated soil and build mounds. The ant mortality or colony elimination in field studies with fipronil granular products was usually not observed for 4–8 wk after application (Barr 2004). To compare the concentrations of fipronil in this experiment to those in the field, two assumptions were made: 1) fipronil will penetrate 1.0 cm depth of soil and 2) weight of 1.0 cm top soil is 1.0 g/cm^3. Under these assumptions, the concentration of fipronil in the soil is 0.1006 and 0.1396 ppm for Over'n Out and TopChoice, respectively, and both are much lower than the LC_{50} values observed in this experiment. This may explain why mortality or colony elimination in field studies with these products was not observed for 4–6 wk after treatment.

To exploit digging behavior, in addition to lack of repellency, the insecticide must also be slow acting so that worker ants do not die after the initial contact. A similar concept has been used for termite control. Nonrepellent and slow-acting termiticides had greater impact on termite population than repellent insecticides by allowing termites to move within treated soil and to transfer nonrepellent termiticides among termites (Kard 2001, Shelton and Grace 2003). Indeed, fipronil is one of the nonrepellent termiticides. Although fipronil has been used to control fire ants, this is the first study that reports on the effect of digging behavior on the efficacy of contact insecticides against fire ants.

Another potential way to exploit digging behavior in fire ant management is to use chemicals that elicit or enhance the digging behavior in the contact insecticide formulations, such as chemical releaser of digging behavior. Although no chemical releaser of digging behavior of *S. invicta* has been identified, there is evidence of the existence of such chemicals in other ant species. Hangartner (1969) reported that carbon dioxide is a releaser for digging behavior in *S. geminata.* Blum and Warter (1966) identified 2-heptanone as a chemical releaser of ant digging behavior for *Conomyrma pyramica* Say. Wilson (1958) reported that workers of *Pogonomyrmex badius* (Latreille) began to show digging behavior after being exposed to mandibular gland secretion. He also demonstrated that many volatile chemicals induced behavior patterns similar or identical to those released by the mandibular gland secretion. For example, workers responded immediately with alarm behavior and in time with digging behavior after they directly contact filter paper treated with small amount of formic acid, ethylamine, n-butyric acid, or n-caproic acid. Hubbard (1974) reported that *S. invicta* workers dug preferentially in their own nest material, indicating the existence of chemicals in the nest, which may be either digging releasers or enhancers. However, more research is needed to investigate chemical releasers of digging behavior in *S. invicta.*

Exploiting ant digging behavior will make using contact insecticide more environmentally friendly, because less insecticide is needed to achieve the same level of control than conventional insecticide formulations.

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References Cited

Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants. U.S. Dep. Agric. SEA Adv. Agric. Tech. AAT-S-21/April 1981.

Barr, C. L. 2004. Speed of suppression of different carrier formulations of fipronil. Result demonstration handbook 1999–2003, Texas Agric. Ext. Serv., Bryan, TX. (http://fireant.tamu.edu).

Barr, C. L., and R. L. Best. 2004. Comparison of different formulations of broadcast fipronil for the control of red imported fire ants. Result demonstration handbook 1999–2003, Texas Agric. Ext. Serv., Bryan, TX. (http://fireant.tamu.edu).

Blum, M. S., and S. L. Warter. 1966. Chemical releasers of social behavior. VII. The isolation of 2-heptanone from *Conomyrma pyramica* (Hymenoptera: Formicidae: Dolichoderinae) and its modus operandi as a releaser of alarm and digging behavior. Ann. Entomol. Soc. Am. 59: 774–779.

Collins, H. L., and A.M.A. Callcott. 1998. Fipronil: an ultra-low-dose bait toxicant for control of red imported fire ants (Hymenoptera: Formicidae). Fla. Entomol. 81: 407–415.

Costa, H. S., and M. K. Rust. 1999. Mortality and foraging rates of Argentine ant (Hymenoptera: Formicidae) colonies exposed to potted plants treated with fipronil. J. Agric. Urban Entomol. 16: 37–48.

Deneubourg, J. L., and N. R. Franks. 1995. Collective control without explicit coding: the case of communal nest excavation. J. Insect Behav. 8: 417–432.

Francke, O. F. 1983. Efficacy tests of single-mound treatments for control of red imported fire ants, *Solenopsis invicta* Buren. Southwest. Entomol. 8: 42–45.

Greenberg, L. Reierson, D., and M. K. Rust. 2003. Fipronil trials in California against red imported fire ant, *Solenopsis invicta* Buren, using sugar water consumption and mound counts as measures of ant abundance. J. Agric. Urban Entomol. 20: 221–233.

Grosman, D. M., W. W. Upton, F. A. McCook, and R. F. Billings. 2002. Attractiveness and efficacy of fipronil and sulfluramid baits for control of the Texas leafcutting ant Southwest. Entomol. 27: 251–256.

Hangartner, W. 1969. Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). Psyche 76: 58–67.

Hays, S. B. 1988. History of IFA control measures, pp. 85–90. In S. B. Vinson and J. Teer [eds.], The imported fire ant: assessment and recommendation. Proceedings of Governor's Conference, Sportsmen Conservations of Texas, Austin, TX.

Hooper-Bui, L. M., and M. K. Rust. 2000. Oral toxicity of abamectin, boric acid, fipronil, and hydramethylnon to laboratory colonies of Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 93: 858–864.

Hubbard, M. D. 1974. Influence of nest material and colony odor on digging in the ant *Solenopsis invicta* (Hymenoptera: Formicidae). J. Ga. Entomol. Soc. 9: 127–132.

Kard, B. 2001. Gulfport studies stay the course. Pest Control 69: 30–33.

Lopez, R., D. W. Held, and D. A. Potter. 2000. Management of a mound-building ant, *Lasius neoniger* Emery, on golf putting greens and tees using delayed-action baits or fipronil. Crop Sci. 40: 511–517.

Markin, G. P., J. O'Neil, and J. Dillier. 1975. Foraging tunnels of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). J. Kans. Entomol. Soc. 48: 83–89.

Mikheyev, A. S., and W. R. Tschinkel. 2004. Nest structure of the ant *Formica pallidefulva:* costs and rules of excavation. Insectes Soc. 51: 30–36.

Morrill, W. L. 1997. Red imported fire ant control with diazinon and chlorpyrifos drenches. J. Ga. Entomol. Soc. 12: 96–100.

Rasse, P., and J. L. Deneubourg. 2001. Dynamics of nest excavation and nest size regulation of *Lasius niger* (Hymenoptera: Formicidae). J. Insect Behav. 14: 433–449.

Rhoades, W. C., and D. R. Davis. 1967. Effects of meteorological factors on the biology and control of the imported fire ant. J. Econ. Entomol. 60: 554–558.

SAS Institute. 1999. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.

Shelton, T. G., and J. K. Grace. 2003. Effects of exposure duration on transfer of nonrepellent termiticides among workers of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96: 456–460.

Sudd, J. H. 1969. The excavation of soil by ants. Z. Tierpsychol. 26: 257–276.

Sudd, J. H. 1971. The effect of tunnel depth and of working in pairs on the speed of excavation in ants (*Formica lemani* Bondroit). Anim. Behav. 19: 677–686.

Sudd, J. H. 1972. The response of digging ants to gravity. Insectes Soc. 19: 243–250.

Sudd, J. H. 1975. A model of digging behaviour and tunnel production in ants. Insectes Soc. 22: 225–235.

Ulloa-Chacón, P., and G. I. Jaramillo. 2003. Effects of boric acid, fipronil, hydramethylnon, and diflubenzuron baits on colonies of ghost ants (Hymenoptera: Formicidae). J. Econ. Entomol. 96: 856–862.

Valles, S. M., and S. D. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of *GP*-9 alleles. Insectes Soc. 50: 199–200.

Vargo, E. L., and S. D. Porter. 1989. Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 82: 307–313.

Williams, D. F., and C. S. Lofgren. 1983. Imported fire ant (Hymenoptera: Formicidae) control: evaluation of several chemicals for individual mound treatments. J. Econ. Entomol. 76: 1201–1205.

Wilson, E. O. 1958. A chemical releaser of alarm and digging behavior in the ant *Pogonomyrmex badius* (Latreille). Psyche 65: 41–51.

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